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# Study of Biomass Feedstocks from Poplar Wood Using Supercritical Fluids

by

E.C. McDonald, B. Bennet and J. Howard
B.C. Research
Vancouver, B.C.

Contractor: B.C. Research
Chemical Technology Division
3650 Westbrook Mall
Vancouver, B.C. V6S 2L2

Scientific Authority:

Mr. R.J. Neale
ENFOR Program Co-ordinator, Biomass Conversion
Canadian Forestry Service
Environment Canada
351 St. Joseph Blvd.
Hull, Quebec K1A 1G5

Technical Liaison Officer:

Dr. J.F.G. Mackay
Research Scientist, Energy from Wood Waste
FORINTEK Canada Corp.
Western Laboratory
6620 N.W. Drive
Vancouver, B.C. V6T 1X2

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# STUDY OF BIOMASS FEEDSTOCKS FROM POPLAR WOOD USING SUPERCRITICAL FLUIDS

ENFOR Project C-295

## ABSTRACT

Pretreatment of poplar wood with either sulfur dioxide or ammonia prior to enzymatic hydrolysis gave substantial increases in the yields of reducing sugars. As a result of chemical modification to the wood lignin-carbohydrate association, yields of up to 55% were achieved at pressures below the critical pressures of these gases. Pretreatment with ammonia or sulfur dioxide in their supercritical states neither increased the yields of sugars nor decreased the contact times. Enzymatic hydrolysis of ammonia and sulfur dioxide pretreated woods gave yields of 40% as glucose. Sulfur dioxide pretreated wood gave an additional yield of 15% reducing sugars resulting from acid hydrolysis of the wood. Sulfur dioxide and ammonia pretreatments appear to offer alternatives to steam explosion which is currently being given serious consideration as a commercial pretreatment process.

Pretreatment with supercritical propane achieved a modest increase in the yield of reducing sugars obtained by enzymatic hydrolysis of poplar wood. Supercritical carbon dioxide and ethylene pretreatments did not increase the accessibility of carbohydrates to enzymatic hydrolysis.

Acetylation of an ammonia pretreated wood gave a cellulose acetate yield of 80%, almost double that obtained from untreated poplar wood.

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## ABSTRACT OF THE ABSTRACT

A Tear-Sheet Annotation

Enzymatic hydrolysis of both sulfur dioxide and ammonia pretreated poplar wood gave high sugar yields. Supercritical carbon dioxide, ethylene and propane pretreatments gave low sugar yields.

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## STAFF

Dr. John Howard, Manager of the Wood and Industrial Chemistry Section, was program manager for ENFOR Project C-295.

Pretreatments of wood samples with compressed gases, reducing sugar analyses and report preparation were carried out by Ms. Bridie Bennett and Ms. Elizabeth McDonald. Mr. Ernie Lee, Bio-Engineering Division, carried out the enzymatic hydrolysis of the pretreated wood samples.

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- Mr. D. Brown, Stake Technology, for providing a sample of Procell; steam exploded poplar.

- Dr. G. Mackay, Forintek Canada Corp. (Western Laboratory), for obtaining the poplar tree trunk used in this work and for his support as our Technical Liaison Officer.

# 1.0 INTRODUCTION

## 1.1 Objective

To increase the accessibility of poplar (Populus tremuloides) carbohydrates to sugar producing cellulase enzymes by treatment of wood with supercritical fluids.

## 1.2 Background

The use of biomass resources to produce liquid and gaseous fuels, chemical feedstocks and thermal energy is viewed as a means for reducing our dependence on imported oil and for using excess or underutilized biomass materials. If a successful cellulose hydrolysis process was available, the production of wood sugar could form the basis of new enterprises providing an important source of chemicals and fuels.

While there has been considerable progress in the study of enzymatic hydrolysis of cellulose, the key factor is the pretreatment required to permit access of the enzyme to the carbohydrates. The applications of enzymatic hydrolysis techniques have been limited by a lack of an economical pretreatment. Without such pretreatment, hydrolysis is slow and incomplete. Cellulose has a high degree of crystallinity which permits only low accessibility to enzymes, and the lignin, interspersed among the fibrous constituents, greatly inhibits enzymatic hydrolysis (Saeman, 1981).

Pretreatment of wood to enhance enzymatic hydrolysis generally fits into one of three categories: chemical, biological or physical. Chemical pretreatment has included the use of sodium hydroxide, ammonia, concentrated sulfuric and hydrochloric acids, cupram, cuen and cadoxen. Biological pretreatment of wood has been primarily concerned with the use of fungi with different enzyme complements. Physical pretreatment includes ball-milling, irradiation and steam explosion (Saddler, 1982).

The pretreatment of wood using supercritical fluids was proposed by B.C. Research as a novel physical pretreatment which should be explored as an alternative to those already investigated.

Gases which are compressed above their critical pressure ($P_c$) and heated above their critical temperature ($T_c$) become supercritical fluids. These fluids exhibit characteristics of a liquid while having the mobility of a gas. The extremely low viscosities of supercritical fluids compared to liquids confers upon them the unique ability to penetrate substances such as wood, which are normally impermeable to liquids. When the pressure on a supercritical fluid was released rapidly, two effects on the wood were anticipated. First, on a macro scale, there would be an explosive or bursting effect on the wood cells. Second, on a molecular level, there would be removal and redistribution of substances formerly dissolved or swollen by the supercritical fluid.

A pretreatment which increased the accessibility of carbohydrates to enzymes could be expected, also, to allow greater access of acetyl groups to cellulose. Therefore, an increased yield of cellulose acetate should be obtained from pretreated poplar wood.

The material chosen for supercritical fluid pretreatments was poplar wood, trembling aspen (Populus tremuloides). Poplar is probably the most common and widely distributed tree in North America. Since it is very susceptible to decay, poplar is usually harvested before the tree reaches the optimum size for the manufacturing of plywood and lumber. The susceptibility to decay is associated with the low lignin content of poplar (17%), which is lower than most hardwoods (20 to 26%) and all softwoods (30 to 35%). Trembling aspen accounts for about 80% of the merchantable poplar volume in Canada and only a small proportion of the allowable cut is utilized (Neilson, 1975).

## 2.0 MATERIALS AND METHODS

### 2.1 Preparation of Wood Samples

A portion of a poplar (Populus tremuloides) tree trunk, harvested from the Slave Lake area in Alberta, was obtained from Forintek Canada Corp., Western Laboratory. The log was 152 cm long with a diameter of 14 cm. A piece 15 cm long was cut from each end and discarded, then the bark removed from the remaining log by hand. Two sides of the log were trimmed to provide flat surfaces so that the log could be fed into a thickness planer. The thickness planer was thoroughly cleaned using compressed air and the cutting surfaces were washed with acetone. Shavings were collected from the planer in plastic bags, the bags were sealed and the shavings stored at -10°C. Shavings from the outer 1 to 2 cm of the radius of the log were discarded, the shavings from the next 2 to 3 cm were collected and the core discarded. The shavings were of varying dimensions, ranging from large pieces, 5 cm by 2 cm by 1 mm, to small pieces, 5 mm by 2 mm by 1 mm. Subsamples for treatment were removed after thorough mixing of the shavings.

The poplar shavings had a moisture content of about 50% and an elemental composition based on dry wood, of: carbon, 46.44%; hydrogen, 6.15%; oxygen, 47.31%; nitrogen, 0.10% and sulfur, less than 0.05%.

The sample of Douglas-fir wood was prepared using the same procedure. The Douglas-fir wood was obtained from MacMillan Bloedel Ltd, Vancouver Plywood Division. The Douglas-fir shavings had a moisture content of about 28%.

## 2.2 Pretreatment of Poplar Wood with Compressed Gases

### 2.2.1 Carbon Dioxide, Ethylene and Nitrogen

The equipment shown schematically in Figure 1 was used for the pretreatment of poplar wood shavings with gases having cylinder pressures in excess of 5 MPa. A 76 MPa double-ended, motor driven diaphragm compressor (Superpressure Inc.) was controlled by a pressure limiting meter (Superpressure Inc.) which was located between the compressor and the base of a Kuentzel pressure vessel (Autoclave Engineers Inc.). The top of the vessel was connected to a valve (Autoclave Engineers Inc.).

A stainless steel screen was placed at the bottom of the pressure vessel, and the vessel filled with 25 g of lightly compacted, wet shavings. Glass wool and a second screen were placed on top of the wood. The sealed vessel was placed in the preheated water bath and the system pressurized. At the end of the run the compressor was turned off and the pressure released rapidly. Usually about 20 seconds was required to reach atmospheric pressure. The treated sample was freeze-dried for 16 hours and stored in a sealed plastic bag at -10°C prior to treatment with the enzyme.

Entrainers were added to some of the supercritical carbon dioxide runs. The liquids or aqueous solutions were mixed with Celite 545, using just sufficient Celite to absorb the liquid. The Celite was placed at the bottom of the pressure vessel on top of a stainless steel screen. About 10 mm of glass wool was placed on top of the Celite, then 25 g of wet shavings added to the vessel. Glass wool and a screen were placed at the top of the vessel. The wood sample was then treated in the sealed vessel and subsequently freeze-dried, as described above.

2.2.2 Ammonia, Sulfur Dioxide and Propane

For gases with a cylinder pressure less than 5 MPa, a high pressure liquid pump was used to transfer the liquefied gas to the reaction vessel. The equipment, shown schematically in Figure 2, comprised a 34 MPa liquid metering pump (Milton Roy Co.) connected by a preheating coil to the base of a Kuentzel pressure vessel (Autoclave Engineers Inc.). The pressure was released through a valve (Autoclave Engineers Inc.) at the top of the vessel. A fluidized sand bath (Techne Inc.) was used to heat the sample and gas. The inverted gas cylinder was connected to the pump and the pump valve housing was covered with dry ice contained by aluminum foil (to prevent vapour locks).

The pressure vessel was filled with 25 g of lightly compacted, wet shavings, and connected to the pump. The pump was turned on and the vessel lowered into the preheated sand bath. The pressure was maintained by turning the pump on and off, as needed. The reaction time was measured from the time the vessel was lowered into the bath. At the completion of the run the pump was turned off, the valve was fully opened to release the pressure and the vessel was removed from the sand bath. About 20 seconds was required to reach atmospheric pressure. The treated sample was freeze-dried for 16 hours and stored in a sealed plastic bag at -10°C prior to treatment with the enzyme.

For low pressure, subcritical runs at 340 kPa the autoclave was connected to the coil, the cylinder valve opened briefly, the vessel lowered into the bath and the pressure maintained by opening either the cylinder valve or the pressure release valve. With sulfur dioxide, the cylinder valve was left open once the vessel was in the sand bath and the pump used to maintain a pressure of 340 kPa.

## 2.3 Enzymatic Hydrolysis with Cellulase

### 2.3.1 Preparation of Sodium Acetate Buffer

Acetic acid solution (0.2 M) was prepared by diluting 11.55 ml glacial acetic acid to 1000 ml with distilled water. Sodium acetate solution (0.2 M) was prepared by dissolving 16.4 g sodium acetate in distilled water and diluting to 1000 ml. The 0.2 M sodium acetate buffer, pH 5.0, was prepared by adding 14.8 ml of acetic acid solution to 35.2 ml of sodium acetate solution and diluting to 100 ml with distilled water (Mackie, 1962).

### 2.3.2 Preparation of Enzyme Solution

Frozen Maxazyme CL 2000, 500 mg, was weighed into a beaker, mixed with 100 ml acetate buffer and 5 mg thymol added to prevent microbial growth. The mixture was warmed to 30°C in a water bath and stirred for 10 minutes. The solution was filtered through a glass fibre filter by vacuum filtration and the filtrate used for the enzymatic hydrolysis test.

The enzyme was provided by the Pulp and Paper Research Institute of Canada and was originally obtained from Gist Brocades nv.

### 2.3.3 Hydrolysis with Cellulase

The freeze-dried wood samples were ground to pass a screen of pore size 0.5 mm (35 mesh). The samples, 50 mg, were weighed into 50 ml Erlenmeyer flasks and incubated with 5 ml enzyme solution for 24 hours at 30°C. The flasks were shaken continuously.

At the end of the incubation, the hydrolyzates were centrifuged at 6500 gravity units for 15 minutes. The supernatants were analyzed for reducing substances as described in Section 2.4.2.

Samples of ground, freeze-dried wood, 50 mg, were carried through the same procedure, excluding the Maxazyme solution, but with the addition of 5 ml of 0.2 M sodium acetate buffer. This experiment gave the reducing substances present in the sample before enzymatic hydrolysis. A sample of enzyme solution and buffer were also carried through the entire procedure to provide blanks for enzyme activity and reagent absorbance.

The moisture correction factor for the sample, using another portion of wood, was determined by heating the sample at 105°C for 16 hours. The moisture correction factor was equivalent to the weight after drying divided by the weight before drying and was usually about 0.9 for the freeze-dried poplar wood samples.

## 2.4 Determination of the Reducing Substances

### 2.4.1 Preparation of Dinitrosalicylic Acid Reagent

Dinitrosalicylic acid reagent was prepared by dissolving 10.6 g of 3,5-dinitrosalicylic acid and 19.8 g sodium hydroxide in 1416 ml distilled water. To this solution were added 306 g sodium potassium tartrate, 7.6 ml of 90% phenol in water, and 8.3 g sodium metabisulfite.

### 2.4.2 Analysis of the Reducing Substances

Reducing substances were determined in the hydrolysis supernatants by the dinitrosalicylic acid method using glucose as a standard (Miller, 1959). One ml of supernatant was pipetted into a screw capped test tube and to this were added

1 ml of distilled water and 2 ml of dinitrosalicylic acid reagent. The test tube was sealed, the contents mixed, and the tube heated in a boiling water bath for 15 minutes. The contents were diluted with 16 ml of distilled water and mixed well. Absorbances of the samples, glucose standards and enzyme, buffer and wood blanks were read at 575 nm using distilled water as a reference. The reducing substances present in the samples were estimated from a calibration curve of glucose concentration versus absorbance, and recorded as % glucose.

A correction was applied to the sample absorbance for enzyme activity and reagent absorbance. The absorbances of the enzyme and buffer blanks were subtracted from the sample absorbance. This value for reducing substances gave the amount of sugars present in the wood before hydrolysis, plus the sugars produced by enzymatic hydrolysis. This value is called the "Total Reducing Substances".

A second correction was then applied to the sample absorbance for the reducing substances present in the wood prior to hydrolysis. The absorbances of the wood, enzyme and buffer blanks were subtracted from the sample absorbance. This value gave the amount of sugars produced by enzymatic hydrolysis and is called the "Net Reducing Substances".

Both values were calculated as milligrams glucose per 100 mg dry wood and recorded in the tables as "% glucose". Frequent mention in the text to glucose yield refers strictly to the percent yield of reducing substances which will include xylose and other reducing sugars.

## 2.5 Procell Sample

The sample of steam exploded poplar was provided by Stake Technology in February, 1980. Process details were not revealed to B.C. Research. Information from various references indicated that the reaction temperature was probably in the range 200 to 240°C, the reaction time was several minutes and the steam pressure was between 2 and 3 MPa.

## 2.6 Hurricane Process Sample

The sample was prepared at the Pulp and Paper Research Institute of Canada. The process details were provided with the sample. Poplar wood chips were dried at 375°C for 10 minutes, then transferred to a cyclone mill where the chips were milled to a powder. The accessibility of carbohydrates to enzymatic hydrolysis was further increased by ball-milling the wood powder for about 10 days.

## 2.7 Preparation of Cellulose Acetate

Four g of the freeze-dried wood sample was soaked in water for 1 hour with occasional stirring, then vacuum filtered. The "re-expanded" wood sample was slurried with three successive portions of 30 g glacial acetic acid. Each portion of acetic acid was shaken with the sample for 1 hour before removal by vacuum filtration. The resulting water-free pad was treated with 41 g of a mixture consisting of 200 g glacial acetic acid, 80 g acetic anhydride, 6.3 g concentrated sulfuric acid and 1.8 g sodium acetate, cooled initially to 0°C. The acetylation mixture was stirred in a glass reaction vessel held at 38°C for 15 minutes, during which time a brown coloured acid dope solution was formed. A solution, 31.2 g, comprising 200 g glacial acetic acid, 50 g water and 2.3 g sodium acetate was

then added and the resulting partial hydrolysis reaction allowed to take place for 19 hours at 53°C. Insoluble lignin material was then removed by centrifugation at 650 gravity units after reducing the dope viscosity by addition of 10 g acetic acid. After decanting the supernatant, the sediment was further washed by re-slurrying and centrifuging from another 20 g of acetic acid. The combined supernatant dopes were centrifuged again to remove fine particles. The cellulose acetate product was then precipitated and hardened by addition of 400 g of 0.1% calcium acetate solution. The slurry was filtered through a glass fibre filter, washed with 50 ml of water and the cellulose acetate product dried at 105°C for 16 hours. The yield of product was expressed as a percentage of the original dry weight of wood.

This procedure was devised by one of the authors, John Howard, based on his experience in the field of cellulose acetate chemistry.

## 3.0 RESULTS AND DISCUSSION

Highlights of the results are presented in Table 1, showing the effect of pretreatment of poplar wood with compressed gases on the yield of reducing sugars by enzymatic hydrolysis. For comparison, the results obtained with untreated wood, Procell and Hurricane Process samples are also included. Of the compressed gas pretreatments, sulfur dioxide and ammonia pretreatments were the most effective in increasing the accessibility of carbohydrates to enzymatic hydrolysis.

The following sections discuss, in detail, the results obtained with the gas pretreatments.

### 3.1 Pretreatment with Nitrogen

Treatments of poplar wood were carried out using nitrogen at 40°C and 62 MPa. These runs were performed to see if a compressed gas, which was not in its supercritical state, would achieve a disruption of the cell wall simply because of the pressure applied to the wood. The yield of reducing sugars increased from 9.5% for untreated wood to 12% for wood pretreated with nitrogen at a pressure of 62 MPa. Therefore, a small increase in the yield of reducing sugars by enzymatic hydrolysis was achieved by the pretreatment of poplar wood with a gas at high pressure.

### 3.2 Pretreatment with Carbon Dioxide

Results of the pretreatment of poplar wood with supercritical carbon dioxide ($T_c$ = 31°C, $P_c$ = 7.4 MPa) are shown in Table 2. The highest yield of reducing sugars after enzymatic hydrolysis was 12%. This small increase can be entirely attributed to the application of pressure to the wood.

The evidence which suggested that supercritical carbon dioxide could cause a disruption to the cell wall was obtained in previous work using supercritical fluids to extract resin acids from southern pine (McDonald, 1982). A large increase in the amount of resin acids extracted by hexane was found after supercritical carbon dioxide extraction of the wood. A hexane extraction of pine sawdust for 6 hours gave a resin acids yield of 11.5%, based on oven dried wood. A supercritical carbon dioxide extraction gave a resin acids yield of 5.6%, and a hexane extraction of the residue from this run gave a further yield of 19.1%.

Pretreatment with supercritical carbon dioxide clearly causes some disruption in the cell wall structure, but the effect is not sufficient to permit access to the carbohydrates of the large enzyme molecule. The much smaller hexane molecule, on the other hand, can penetrate previously inaccessible areas following treatment with supercritical carbon dioxide. Soxhlet extractions of treated and untreated poplar, unlike southern pine, did not show an increase in the weight of extractives.

In experiments to extract chemicals from various substrates with supercritical carbon dioxide, the addition of a second substance, an entrainer, has often been found to enhance the solubility of chemicals in the fluid. Therefore, in an attempt to enhance the disruption to the cell structure, runs were carried out in which entrainers such as ethanol, ammonium hydroxide and formic acid were added. The results, presented in Table 3, show that ethanol was the only entrainer which increased the accessibility of carbohydrates to the enzyme. The yield of reducing sugars by enzymatic hydrolysis of the supercritical carbon dioxide/ethanol pretreated sample was 16%. However, this increase was not sufficient to indicate that further work using supercritical carbon dioxide would prove to be of value.

## 3.3 Pretreatment with Propane

Pretreatment of poplar wood with supercritical propane ($T_c$ = 96.8°C, $P_c$ = 4.3 MPa) increased the yield of reducing sugars to about 17%, from 9.5% for the untreated wood. The results are presented in Table 4.

The reaction temperature used for the propane pretreatments, 110°C, was thought to be a factor in the increased yield of reducing sugars. However, wood samples placed in a test tube and heated in the sand bath at 110°C for 40 minutes gave no increase in the yield of sugars produced by enzymatic hydrolysis. Also, an increase in yield was not observed when supercritical carbon dioxide pretreatment was carried out at 90°C rather than 40°C (Table 2). Therefore, it appears that the larger size of the propane molecule alone was probably the main factor responsible for the increased yield of reducing sugars. The elevated temperature may have caused a slight degree of wood "softening" which permitted propane to enter the lignocellulosic structure more readily. On release of the pressure, the propane expanded rapidly and caused some disruption to the cell walls, thereby permitting greater enzymatic hydrolysis. However, given the high pressure required for this modest increase in glucose yield, further work with propane did not appear to be justified.

## 3.4 Pretreatment with Ethylene

Treatment of poplar wood with supercritical ethylene ($T_c$ = 9.9°C, $P_c$ = 5.1 MPa) increased the reducing susbstances to 11%, from 9.5% for the untreated wood. These runs were carried out at 15°C and 62 MPa, and the increase can be attributed to the effect of pressure applied to the wood.

## 3.5 Pretreatment with Anhydrous Ammonia

The fact that liquid ammonia exerts a strong swelling action on wood and cellulose and can effect a phase change in the crystal structure of cellulose has long been recognized. Poplar appears to be unique in the extent of its response to ammoniation (Millett, 1975).

Intracrystalline swelling involves a penetration of the crystalline as well as the amorphous cellulose regions. This can lead to new crystalline modifications or, in some cases, to unlimited swelling with complete solution of the cellulose. Anhydrous ammonia is an agent which causes limited swelling and thus provides a pathway toward alteration of cellulose crystalline structure with the possibility of enhanced hydrolytic reactivity (Millett, 1976).

The results of pretreatments of wet poplar wood with anhydrous ammonia at 145°C are presented in Table 5. The yield of reducing sugars was dependent on both the pressure and the reaction time. To obtain a yield of 40% glucose, comparable to the Hurricane Process sample yield (Table 1), either a pressure of 1.4 MPa could be applied to the wood for 120 minutes at 145°C or a pressure of 7 MPa could be applied for 30 minutes at the same temperature. The net and total reducing substances were equal for the ammonia pretreated samples indicating that sugars were not produced during the treatment.

The use of ammonia in its supercritical state ($T_c$ = 132.5°C, $P_c$ = 11.4 MPa) gave a lower yield of reducing substances than the subcritical pretreatments. It was anticipated that supercritical ammonia would give high glucose yields with short reaction times (less than 5 minutes), but this was never achieved.

About 57% of wood substances in poplar are hexosans, which, on hydrolysis could be converted to hexose sugars (Ritter, 1947). The results for the Hurricane Process sample (Table 1) and the ammonia pretreated samples (Table 5) indicate that a glucose yield of about 45% is the maximum yield produced by enzymatic hydrolysis of poplar wood.

The freeze-dried wood had a total nitrogen content of 1.8% and a water insoluble nitrogen content of 0.4%. The nitrogen content of the untreated wood was 0.1%. Therefore, ammonia pretreatment would be advantageous for any biological process requiring the addition of nitrogen.

## 3.6 Pretreatment with Anhydrous Sulfur Dioxide

Since lignin is a major deterrent to the enzymatic utilization of biomass, delignification would appear to provide a solution to this problem. Treatment of moist wood with gaseous sulfur dioxide appears to disrupt the lignin-carbohydrate association in situ without the selective removal of either constituent (Millett, 1976).

Initially, pretreatments were carried out at 165°C to observe the effect of sulfur dioxide in its supercritical state ($T_c$ = 157.8°C, $P_c$ = 7.9 MPa). The results presented in Table 6 show that pretreatment with supercritical sulfur dioxide did not give high yields of reducing sugars by enzymatic hydrolysis. At a pressure of 14 MPa the net yield was 22% glucose. The samples pretreated at 165°C were washed and neutralized prior to enzymatic hydrolysis, consequently sugars produced by acid hydrolysis were removed. The total reducing sugar yield would probably have been considerably greater than the net yield if the samples had not been neutralized.

While the literature (Millet, 1976) recommends that samples of wood contacting sulfur dioxide be washed and neutralized prior to enzymatic hydrolysis, Maxazyme was not inhibited by residual sulfur. The treated, unwashed samples after freeze-drying had a sulfur content of about 0.4%. Therefore, washing was discontinued in later phases of the work.

The wood samples were susceptible to charring when pretreated with sulfur dioxide at 165°C. The temperature of the sand bath could be controlled only between 160 to 170°C and if the temperature was maintained at the upper limit for some time, the combination of temperature and rate of acid hydrolysis was sufficient to destroy the wood. Consequently the reaction temperature was decreased to 120°C for subsequent runs.

Pretreatment of wet poplar wood with anhydrous sulfur dioxide at a temperature of 120°C gave total reducing sugar yields of 28 to 55%, as shown in Table 6. At a pressure of 340 kPa the yield increased from 28 to 43% when the reaction time was increased from 30 to 120 minutes. At a reaction time of 30 minutes, an increase in pressure from 340 kPa to 7 MPa gave an increase in yield from 28 to 55%.

About 57% of wood substances in poplar are hexosans, which, on hydrolysis could be converted to hexose sugars (Ritter, 1947). The pretreatment of poplar wood with sulfur dioxide at 7 MPa and 120°C for 30 minutes has, therefore, achieved the maximum yield of reducing sugars obtainable from poplar wood. Acid hydrolysis of the wood during treatment gave a yield of 16% glucose and subsequent enzymatic hydrolysis of the sample produced 39% glucose, to give a total reducing substances yield of 55%.

The Procell sample also showed a difference between net and total reducing substances, 43 and 50% respectively. The high pressure steaming of moist lignocellulosic substrates results in

the partial decomposition of the hemicellulose components to acids, mainly acetic acid, which catalyse the depolymerization of the hemicellulose and lignin. The sugar yields from the sulfur dioxide pretreated wood compare favourably with the yields from both the steam exploded and ball-milled poplar samples.

## 3.7 Comparison of Gas Pretreatments with Ball-Milling and Steam Explosion Pretreatments

Figure 3 illustrates the results obtained with sulfur dioxide pretreatment, ammonia pretreatment, steam explosion, ball-milling and wiley-milling.

The subdivision of cellulosic materials to a very small particle size markedly enhances their susceptibility to enzymatic attack. A sample of wiley-milled poplar supplied by the Pulp and Paper Research Institute of Canada (PPRIC) gave a yield of reducing substances of 5.4%. When this sample was further processed at PPRIC by ball-milling for 10 days the yield of sugars increased to 44%. However, production of fine wood particles by the commercial techniques used for the manufacture of wood flour (hammer and attrition milling) is expensive (Millett, 1976).

Treatment with high pressure steam represents another approach toward upgrading the carbohydrate availability of lignocellulosic residues. The cleavage of acetyl groups provides an acidic medium conducive to hydrolytic action. Steaming shows a strong species bias; hardwoods respond much more than softwoods, with poplar showing the highest response among hardwoods. The explosion temperature has a positive effect on glucose yields during the enzymatic hydrolysis, with a

temperature of 250°C giving a total reducing sugars yield of about 50%. However, at about this temperature the material becomes progressively darker, thus indicating that thermal decomposition takes place. This increases the danger of producing toxic phenolic compounds (from lignin pyrolysis) which might interfere with the use of exploded wood as a fermentation feed stock (Jurasek, 1979). The process conditions used to prepare the Procell sample are not known to B.C. Research. Procell gave a net reducing substances yield of 43% and total reducing substances yield of 51% expressed as glucose. This indicates that the Procell sample does not contain materials which inhibit enzymatic hydrolysis.

Both sulfur dioxide and ammonia pretreated samples of poplar wood gave reducing sugar yields which compare favourably with the yields obtained for ball-milled and steam exploded samples.

As shown in Table 1, sulfur dioxide pretreatment gave higher yields of sugars than obtained with steam explosion, at a lower temperature but at the expense of a substantially longer reaction time and at twice the pressure. The wood used for a steam explosion process is usually chipped then further comminuted in a hammer mill. The comminution step may not be required for a pretreatment process with sulfur dioxide. Only a detailed economic analysis of the two processes would allow a true comparison to be made, but it is thought that sulfur dioxide pretreatment could be a viable alternative to a process involving steam explosion.

Ammonia pretreatment required longer reaction times than sulfur dioxide pretreatment and the reducing sugar yields were lower. However, ammonia pretreatment would be of interest if the subsequent hydrolysis required the addition of nitrogen as a nutrient for biological growth. Residual nitrogen in ammonia pretreated wood would lessen these nutrient requirements.

## 3.8 Pretreatment of Douglas-fir Wood with Sulfur Dioxide and Ammonia

Treatment of moist wood with gaseous sulfur dioxide in order to disrupt the lignin-carbohydrate association in situ appears to be a pretreatment which is applicable to both hard and softwoods (Millett, 1976).

To verify this statement, pretreatments were carried out using Douglas-fir shavings as the substrate. The results presented in Table 7 show that while sulfur dioxide increased the yield of reducing substances, the modest increase was entirely due to acid hydrolysis, and the susceptibility of the wood to enzymatic hydrolysis did not change.

Ammonia pretreatment of fir was also carried out and, as expected, there was no increase in the yield of reducing substances.

## 3.9 Enzymatic Hydrolysis with Cellulase

The enzymatic hydrolyis procedure developed by Dr. L. Jurasek, Pulp and Paper Research Institute of Canada, proved to be relatively simple and few inexplicable results were obtained. Statistical analysis of the results of tests carried out on untreated poplar wood over a nine month period is shown in Table 8. One batch of ground wood was used as the control throughout the tests. This wood had a mean reducing substances yield of 9.5% glucose, with a standard deviation of 1.1 and variance of 1.2. Different samples of wood were heated in air alongside the sample undergoing pretreatment at temperatures from 110 to 170°C. Table 8 shows that temperature alone had no effect on the yield of reducing substances from untreated poplar wood.

## 3.10 Preparation of Cellulose Acetate

Four samples of treated poplar wood were acetylated. The cellulose acetate yields are shown in Table 9. Ammonia treated wood gave a significantly greater yield of cellulose acetate (80%) than the yield obtained with untreated wood (47%) and wood pretreated with nitrogen, carbon dioxide or sulfur dioxide.

The swelling effect of ammonia on the cellulose-lignin structure which increases the accessibility of carbohydrates to enzymatic hydrolysis is also seen to increase the degree of acetylation of cellulose in wood.

## 4.0 CONCLUSION

The pretreatment of poplar wood using non-reactive supercritical fluids did not achieve a significant increase in the accessibility of carbohydrates to enzymatic hydrolysis. Pretreatment with supercritical carbon dioxide and ethylene achieved a slight increase in the yield of reducing sugars which was entirely attributable to the application of high pressures to the wood. Pretreatment of poplar wood with supercritical propane achieved a modest increase to 17% glucose from 9.5% for the untreated wood. This increase is attributed to a combination of the reaction temperature, 110°C, which "softened" the wood and allowed penetration of propane into the cell wall, and the relatively bulky propane molecule disrupting the cell wall when the pressure was released.

Sulfur dioxide and ammonia pretreatments of poplar wood achieved considerable increases in the yields of reducing substances. The effect of sulfur dioxide and ammonia was not increased when they were in a supercritical state. Sulfur dioxide at a pressure of 7 MPa, a temperature of 120°C and a reaction time of 30 minutes gave net and total reducing substances yields of 40 and 55% respectively. These results compare favourably with those for steam exploded and ball-milled poplar wood.

Ammonia pretreatment of poplar wood gave lower yields of reducing substances than did sulfur dioxide pretreatment. A yield of 40% glucose was obtained at a pressure of 1.4 MPa, a temperature of 145°C and a reaction time of 120 minutes. This yield compares favourably with that from ball-milled poplar wood.

Ammonia pretreatment was also shown to substantially increase the yield of cellulose acetate. Acetylation of an ammonia pretreated sample gave a cellulose acetate yield of 80%, almost double that obtained from untreated poplar wood.

Sulfur dioxide pretreatment of Douglas-fir gave an increase in the yield of reducing substances, with the increase being entirely caused by acid hydrolysis of the wood and not by an increase in enzymatic hydrolysis.

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FIGURE 1
SCHEMATIC OF EQUIPMENT USED WITH
CARBON DIOXIDE, ETHYLENE AND NITROGEN

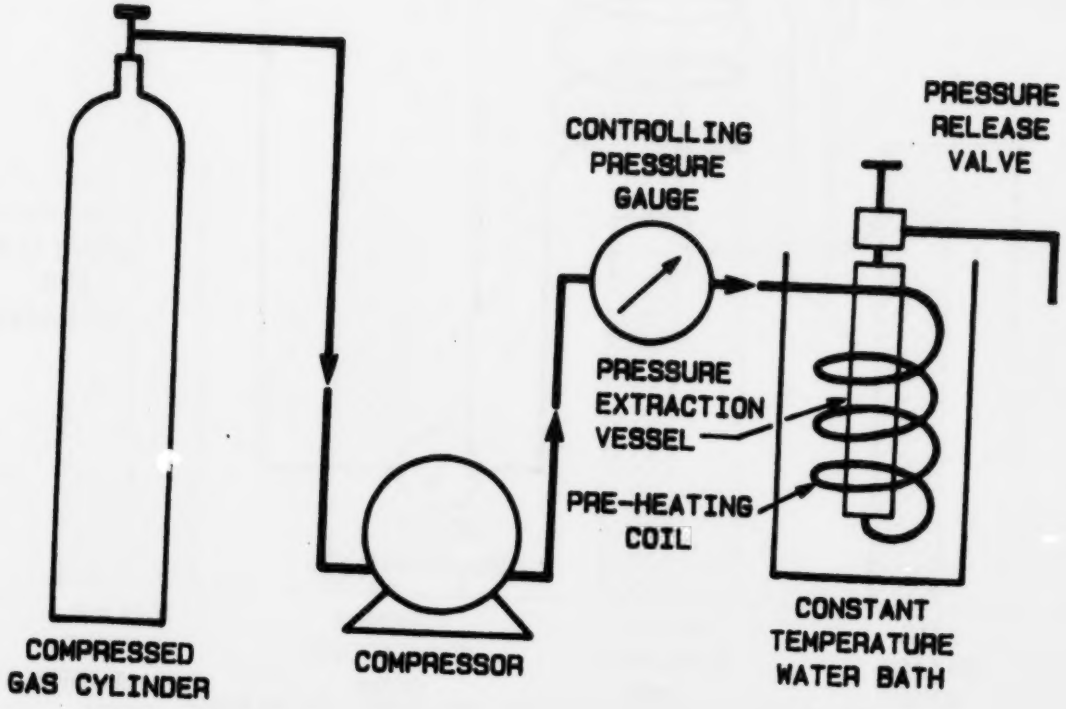

FIGURE 2
SCHEMATIC OF EQUIPMENT USED WITH
AMMONIA, SULFUR DIOXIDE AND PROPANE

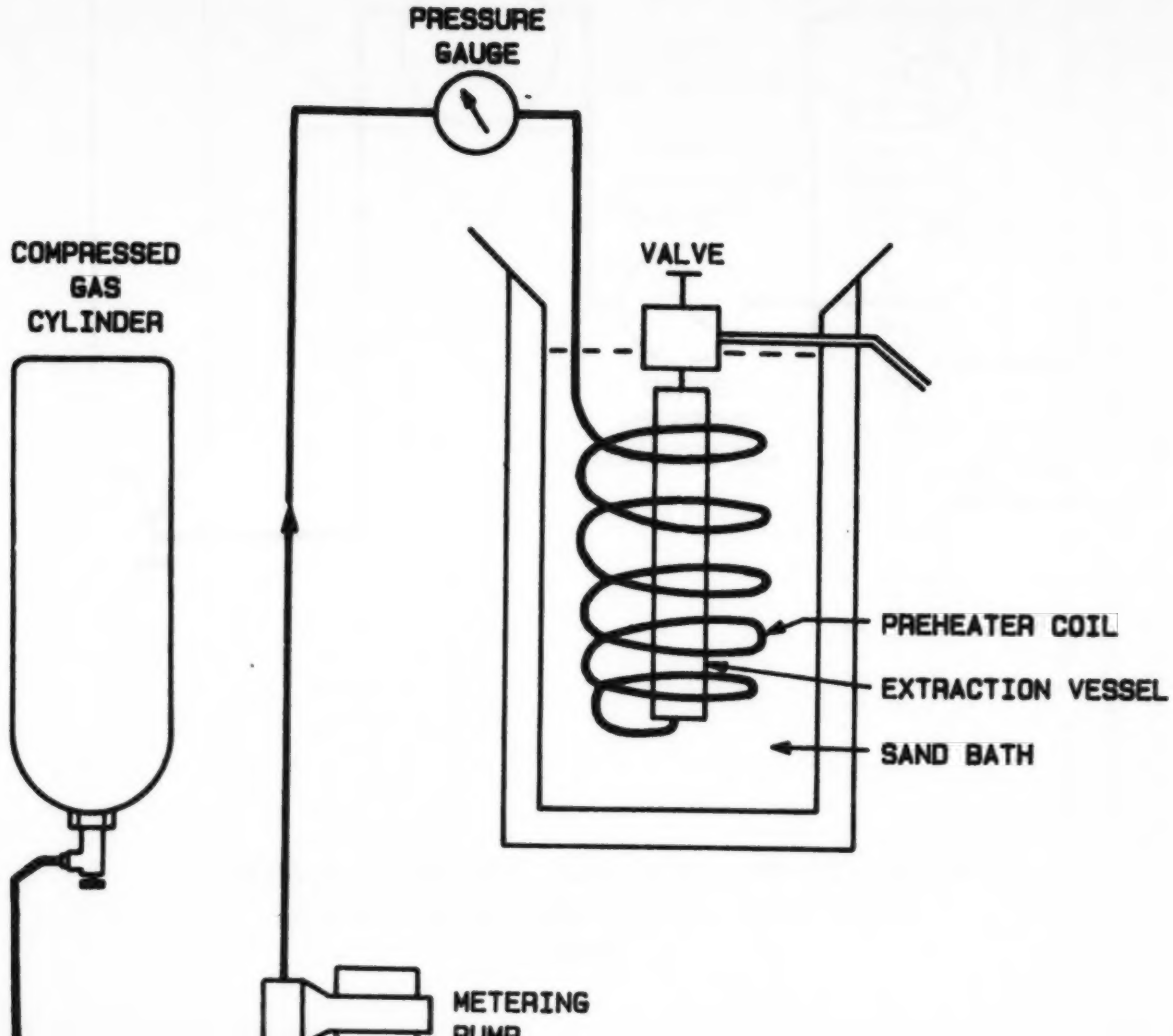

FIGURE 3
COMPARISON OF REDUCING SUBSTANCES YIELDS WITH
GAS PRETREATMENTS, COMMINUTION AND STEAM EXPLOSION

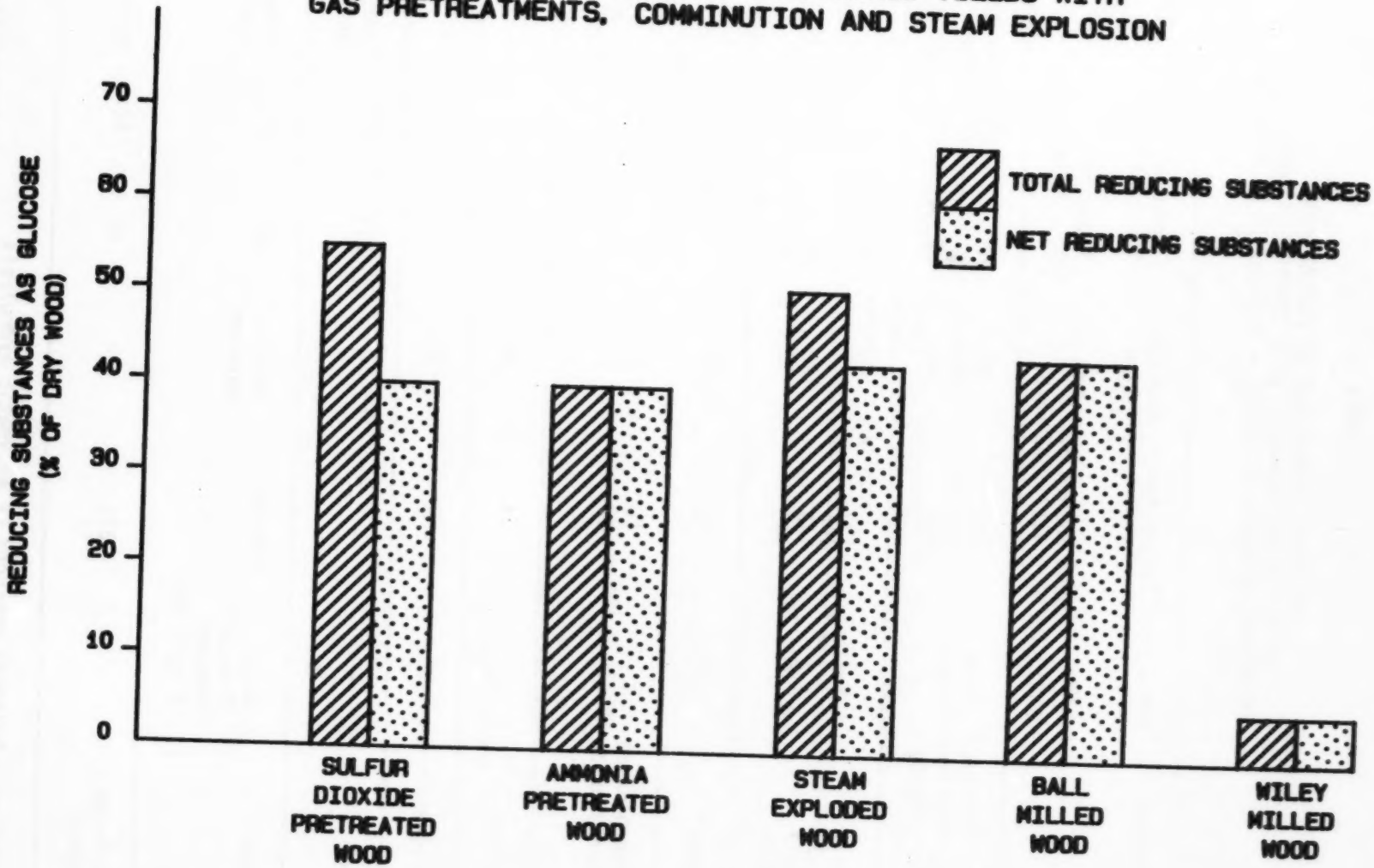

TABLE 1

ENZYMATIC HYDROLYSIS OF POPLAR WOOD:
EFFECT OF PRETREATMENT WITH COMPRESSED GASES
ON YIELDS OF REDUCING SUGARS - HIGHLIGHTS OF RESULTS

| Treatment | Pressure | Temperature | Reaction Time | Reducing Substances | | Comments |
|---|---|---|---|---|---|---|
| | MPa | °C | min | % glucose net[a] | total[b] | |
| Sulfur Dioxide | 0.34 | 120 | 30 | 22 | 29 | Wet shavings use |
| Sulfur Dioxide | 0.34 | 120 | 120 | 30 | 43 | for all compress |
| Sulfur Dioxide | 7 | 120 | 30 | 40 | 55 | gas treatments |
| Ammonia | 0.34 | 145 | 30 | 24 | 24 | |
| Ammonia | 1.4 | 145 | 30 | 33 | 33 | |
| Ammonia | 1.4 | 145 | 120 | 40 | 40 | |
| Ammonia | 7 | 145 | 30 | 40 | 40 | |
| Propane | 7 | 110 | 30 | 17 | 17 | |
| Carbon Dioxide | 62 | 40 | 30 | 12 | 12 | |
| Carbon Dioxide | 62 | 40 | 30 | 16 | 16 | ethanol entraine 40% w/w wet wood |
| Ethylene | 62 | 15 | 15 | 11 | 11 | |
| Nitrogen | 62 | 40 | 15 | 11 | 11 | |
| None | - | - | - | 9.5 | 9.5 | untreated wood: control |
| Procell[c] | Steam Pressure between 2 to 3 MPa | 200-240 | several minutes | 43 | 51 | steam exploded poplar |
| Hurricane Process | - | 375 | 10 | 44 | 44 | ball-milled popl |

[a] reducing substances produced by enzymatic hydrolysis
[b] reducing substances produced by enzymatic hydrolysis and acid hydrolysis
[c] estimate of process conditions from literature sources

TABLE 2

ENZYMATIC HYDROLYSIS OF POPLAR WOOD:
EFFECT OF PRETREATMENT WITH SUPERCRITICAL CARBON DIOXIDE
ON YIELDS OF REDUCING SUGARS

| Temperature[a] °C | Pressure MPa | Reaction Time minutes | Reducing[b] Substances % glucose | Condition of Wood for treatment |
|---|---|---|---|---|
| 40 | 62 | 5 | 12 | wet shavings |
| 40 | 62 | 30 | 12 | wet shavings |
| 40 | 62 | 5 | 12 | dried and ground |
| 40 then raised to 90 during run | 62 | 40 | 10 | wet shavings |
| 90 | 62 | 5 | 11 | wet shavings |
| - | - | - | 9.5 | untreated wood: control |

[a] Carbon dioxide; $T_c$ = 31°C, $P_c$ = 7.4 MPa
[b] Total and net reducing substances equal

TABLE 3

ENZYMATIC HYDROLYSIS OF POPLAR WOOD:
EFFECT OF PRETREATMENT WITH SUPERCRITICAL CARBON DIOXIDE
AND ENTRAINERS ON YIELDS OF REDUCING SUGARS

| Entrainer | Amount of Entrainer added | Condition of Wood for Treatment | Temp. °C | Press. MPa | Reaction Time mins. | Reducing Substances % glucose[a] |
|---|---|---|---|---|---|---|
| Water | 80 ml/100 g dry wood | dry shavings | 40 | 62 | 5 | 9.7 |
| Ethanol | 80 ml/100 g dry wood | dry shavings | 40 | 62 | 5 | 10 |
| Ethanol | 40 ml/100 g wet wood | wet shavings | 40 | 62 | 30 | 16 |
| Formic Acid | 80 ml of 50% formic acid solution/100 g dry wood | dry shavings | 40 | 62 | 5 | 8.8 |
| Formic Acid | 40 ml of 50% solution/100 g wet wood | wet shavings | 40 | 62 | 30 | 10 |
| Ammonium Hydroxide | 80 ml/100 g dry wood | dry shavings | 40 | 62 | 5 | 11 |
| Urea | 16 g/100 g wet wood | wet shavings | 40 | 62 | 30 | 9.7 |
| - | - | untreated wood:control | - | - | - | 9.5 |

[a] Total and Net Reducing Substances equal

TABLE 4

ENZYMATIC HYDROLYSIS OF POPLAR WOOD:
EFFECT OF PRETREATMENT WITH SUPERCRITICAL PROPANE
ON YIELDS OF REDUCING SUGARS

| Temperature[a] °C | Pressure MPa | Reaction Time minutes | Reducing[b] Substances % glucose | Condition of Wood for treatment |
|---|---|---|---|---|
| 110 | 7 | 30 | 17 | wet shavings |
| 110 | 28 | 40 | 15 | wet shavings |
| 110 | 28 | 30 | 9 | dried and ground |
| 110 | 28 | 135 | 18 | wet shavings |
| - | - | - | 9.5 | untreated wood: control |

[a] Propane; $T_c$ = 96.8°C, $P_c$ = 4.3 MPa
[b] Total and Net Reducing Substances equal

TABLE 5

ENZYMATIC HYDROLYSIS OF POPLAR WOOD:
EFFECT OF PRETREATMENT WITH ANHYDROUS AMMONIA
ON YIELDS OF REDUCING SUGARS

| Pressure[a] MPa | Reaction Time mins. | Temperature °C | Reducing[b] Substances % glucose | |
|---|---|---|---|---|
| 0.34 | 30 | 145 | 24 | |
| 0.34 | 120 | 145 | 28 | |
| 1.4 | 30 | 145 | 33 | |
| 1.4 | 90 | 145 | 37 | |
| 1.4 | 120 | 145 | 40 | |
| 3.4 | 30 | 145 | 29 | |
| 7 | 30 | 145 | 40 | |
| 14 | 15 | 145 | 29 | |
| 14 | 30 | 145 | 33 | |
| 28 | 15 | 145 | 31 | |
| - | - | - | 9.5 | untreated wood: control |
| - | 120 | 145 | 8.3 | wood heated in air |

[a] Ammonia; $T_c$ = 132.5°C, $P_c$ = 11.4 MPa
[b] Total and Net Reducing Substances equal

TABLE 6

ENZYMATIC HYDROLYSIS OF POPLAR WOOD:
EFFECT OF PRETREATMENT WITH ANHYDROUS SULFUR DIOXIDE
ON YIELDS OF REDUCING SUGARS

| Pressure[a] MPa | Temperature °C | Reaction Time mins. | Reducing Substances % glucose Net[b] | Total[c] | |
|---|---|---|---|---|---|
| 0.34 | 120 | 30 | 20 | 28[d] | |
| 0.34 | 120 | 120 | 30 | 43[d] | |
| 7 | 120 | 30 | 39 | 55 | |
| 7 | 165 | 15 | 26 | e | |
| 14 | 165 | 15 | 22 | e | |
| 28 | 165 | 15 | 33 | e | |
| - | - | - | 9.5 | 9.5 | untreated wood:control |

a Sulfur dioxide; $T_c$ = 157.8°C, $P_c$ = 7.9 MPa
b Reducing sugars produced by enzymatic hydrolysis
c Reducing sugars produced by acid hydrolysis and enzymatic hydrolysis
d Not washed before enzymatic hydrolysis, glucose produced by acid hydrolysis retained
e Neutralized to pH 7 before enzymatic hydrolysis, glucose produced by acid hydrolysis lost at this stage

TABLE 7

ENZYMATIC HYDROLYSIS OF DOUGLAS-FIR WOOD:
EFFECT OF PRETREATMENT WITH SULFUR DIOXIDE AND AMMONIA
ON YIELDS OF REDUCING SUGARS

| Gas | Pressure MPa | Temperature °C | Reaction Time mins. | Reducing Substances % glucose Net | Total |
|---|---|---|---|---|---|
| Sulfur Dioxide | 0.34 | 120 | 30 | 5.7 | 14 |
| Sulfur Dioxide | 0.34 | 120 | 120 | 5.9 | 17 |
| Control | - | 120 | 30 | 6.3 | 6.3 |
| Ammonia | 14 | 145 | 30 | 5.3 | 5.3 |
| Control | - | 145 | 30 | 3.9 | 3.9 |

TABLE 8

STATISTICAL ANALYSIS OF YIELDS OF REDUCING SUGARS FROM POPLAR WOOD CONTROL SAMPLES

| REDUCING SUBSTANCES, % glucose | | |
|---|---|---|
| Control - same batch of ground wood over a 9 month period | | Different batches of wood heated in air at reaction temperatures from 110 to 170°C |
| 9.71 | | 7.19 |
| 7.40 | | 9.44 |
| 8.39 | | 6.25 |
| 11.08 | | 7.34 |
| 9.52 | | 7.68 |
| 9.70 | | 8.41 |
| 9.51 | | 8.75 |
| 9.36 | | 8.99 |
| 10.05 | | 8.12 |
| 8.53 | | 9.09 |
| 11.04 | | 10.32 |
| | | 8.31 |
| Mean | 9.48 | 8.32 |
| Standard deviation | 1.09 | - |
| Variance | 1.19 | - |

TABLE 9

CELLULOSE ACETATE YIELDS FROM POPLAR WOOD
AFTER TREATMENT WITH COMPRESSED GASES

| Treatment | Pressure MPa | Temperature °C | Cellulose Acetate Yield % of original dry weight |
|---|---|---|---|
| Ammonia | 1.4 | 145 | 80 |
| Sulfur dioxide | 1.4 | 120 | 56 |
| Carbon dioxide | 62 | 40 | 44 |
| Nitrogen | 62 | 40 | 51 |
| Untreated wood | - | - | 47 |